Label – free fluorescence lifetime imaging of microfluidic device based cardiac tissue model metabolism

Rupsa Datta[1], Christopher Heylman[1], David Tran[1], Steven C. George[1], Enrico Gratton[1]
1. Department of Biomedical Engineering, University of California, Irvine.
rupsad@uci.edu

Abstract: In this work, we show mapping of metabolic activity and non-invasive monitoring of drug response of a microphysiological tissue system in a PDMS microfluidic device chamber by fluorescence lifetime imaging of NADH, an endogenous fluorophore.
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1. Summary

There is a growing importance of development of microfludic device based, microphysiological tissue systems which simulates structure and function of human organs. Such tissue chips find application in drug toxicity screening, point-of-care diagnostic devices, targeted drug delivery and so on. Optical imaging proves to be a powerful, non- invasive tool to monitor development of such tissue systems. In this work, we show label-free metabolic imaging of induced pluripotent stem cell derived cardiomyocyte (iPS-CM) 3D spheroids co-cultured with endothelial cells (to form vessel network) and fibroblast in tissue microchamber of a polydimethylsiloxane (PDMS) microfluidic device [1]. We performed fluorescence lifetime imaging microscopy (FLIM) of reduced nicotinamide adenine dinucleotide (NADH), a metabolic coenzyme which plays myriad of roles in cellular oxidation and reduction reactions. NADH is an endogenous fluorophore. Hence this technique does not require staining of the cells to study metabolism, eliminating the need to introduce foreign substance to the biological system. FLIM data was analyzed using the fit-free phasor approach developed at Laboratory of Fluorescence Dynamics, UCI [2]. This allowed us to map free to protein bound NADH ratio distribution in the iPS-CM spheroid and surrounding vessel network, which reflects the metabolic status within the microfluidic device tissue chamber (Figure 1). A higher free to bound NADH indicates glycolysis while a lower ratio indicates oxidative phosphorylation [3]. We also studied the metabolic response of the cardiac tissue model to application of drugs. FLIM of NADH was performed after administration of potassium cyanide (KCN) which is a reducing agent. Phasor analysis of the NADH lifetime distribution showed an increase in free to bound NADH ratio with time, after adding the drug. This reflects a shift of metabolic state from oxidative phosphorylation to glycolysis which corresponds to the fact that KCN blocks cellular respiration causing an increase in free NADH (Figure 2). Hence, in this work, we show mapping of metabolic activity and non-invasive monitoring of drug response of a microphysiological tissue system in a PDMS microfluidic device chamber employing an optical microscopy technique. This method has the advantage of label-free imaging. Also it is non-invasive, thus eliminating the need to extract the cells from the microfluidic device to study metabolism and allowing us to study metabolic activity over time.

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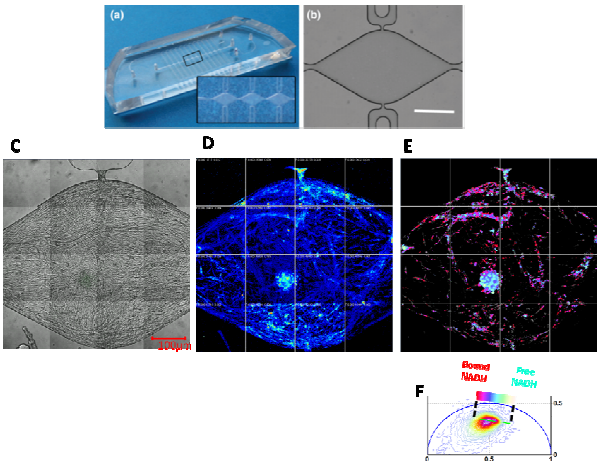

Fig. 1. A) PDMS microfluidic device; B) Tissue chamber of the device [1]; C) Bright field image; D) Average NADH fluorescence intensity image; E) NADH distribution; F) NADH phasor plot.

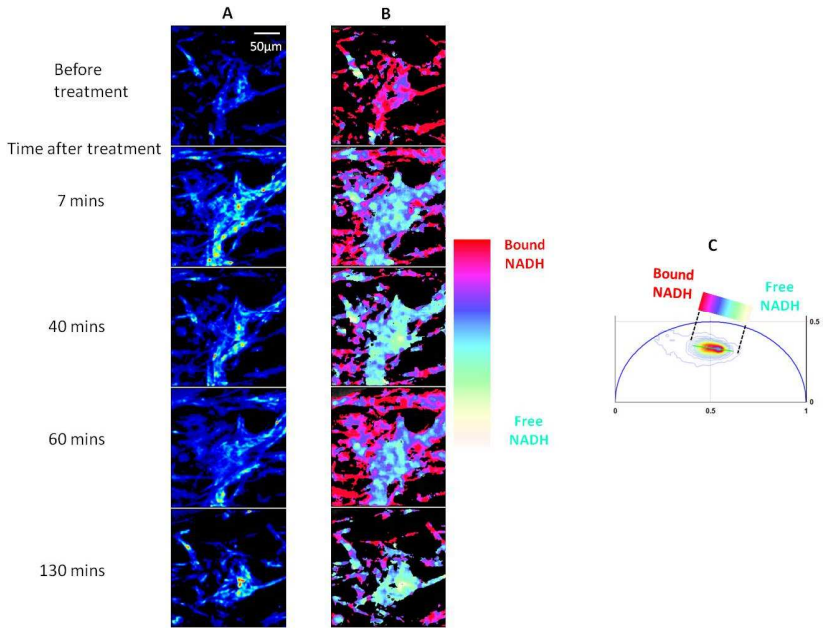

Figure 2. NADH FLIM before and after administration of 4µM KCN. A) NADH fluorescence intensity image; B) NADH distribution showing an increase in free/bound NADH ratio after addition of NADH; C) NADH phasor plot.

2. References

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